United States Department of Agriculture

Animal and Plant Health Inspection Service

Marketing & Regulatory Programs Business Services

4700 River Road
Riverdale, MD
20737

January 14, 2004

Dear Permit Applicants:

This letter is being sent to provide new and updated information on the process for submitting APHIS Form 2000, Application for Permit, when requesting the movement or field release of an organism that is engineered to produce products intended for pharmaceutical use, bioremediation, or industrial nonfood/feed use.

The information on the following pages provides updated guidance from our website and previous letters to applicants. We have also provided new guidance on the biological and safety information that is now required as part of the application process. In addition, we provided new information regarding confinement conditions as a result of two Federal Register Notices published by APHIS in 2003. The newly required information will enable APHIS to conduct the necessary risk assessment to determine the risk associated with the permit request. The information will also serve to provide greater transparency in our processes and address the current level of interest demonstrated by the general public in genetically modified organisms.

The guidance provided on these topics will follow, in order, the items listed on the APHIS Form 2000 permit application, which is included as *Enclosure 1* for your reference.

Below is a list of references for more detailed instructions and information on specific areas of APHIS' regulatory authority and the permitting process:

- For detailed instructions on how to complete APHIS Form 2000, see our *User's Guide for Release Permits* at: http://www.aphis.usda.gov/brs/pdf/usersguide.pdf;
- Additional guidance on how plants expressing pharmaceuticals or biologics will be regulated by APHIS and the Food and Drug Administration (FDA) can be found in the joint *FDA/USDA Draft Guidance for Industry: Drugs, Biologics, and Medical Devices derived from Bioengineered Plants for Use in Humans and Animals* at: http://www.fda.gov/cber/gdlns/bioplant.pdf;
- Information regarding conditions placed on the introductions of plants engineered for production of pharmaceuticals and industrials, beginning with the 2003 growing season is found in our March 10, 2003 Federal Register Notice at: http://www.aphis.usda.gov/brs/pdf/7cfr.pdf;

- Our August 6, 2003 Federal Register Notice at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2003_register&docid=fr06au03-3.pdf is an interim rule that describes what we consider to be a plant-made industrial product and the requirement that introductions of genetically engineered plants that meet this description must occur under an APHIS permit.

1. When Should Permits Be Submitted or Amended?

According to APHIS regulations, permit applications are to be submitted 120 days in advance of the proposed field test release and 60 days in advance of the proposed movement or importation. This time is needed for the biotechnologist to complete the risk assessment and seek concurrence from the State regulatory official. If you are a previous applicant and wish to field test plants engineered for pharmaceutical, industrial, or phytoremediation uses in 2004, you must submit an application for a new permit if you have not already done so.

Pending and current permits for the 2004 growing season can be amended, with the exception of adding new states, new recipient organisms, or new plantings that extend into the next growing season. Amendments may be granted for example, to add new genetic constructs, add additional field sites within a state already listed on the permit, change disposal methods, change the proposed harvest date, etc. Send your request to amend the permit in a letter to the Permit Specialist, Linda Lightle. If you are amending the duration of introduction to extend the harvest date beyond that indicated in Item 8 on the original permit application, you will also need to re-submit the first page of the APHIS form 2000 with Item 8 changed to reflect the extended date.

After the amendment is granted, you will receive a copy of the signed revised APHIS form 2000 to show that you have a valid permit for that time frame. You will need a valid APHIS form 2000 when applying to the FDA or APHIS' Center for Veterinary Biologics. You may wish to notify the BRS biotechnologist in advance that you are sending the amendment. Please state clearly the proposed changes. Be sure to include the permit number on all correspondence and follow the instructions for confidential business information (CBI).

2. Additional Guidance on Required Biological Information

The information presented in 7 CFR 340.4 (b)(5) states that a description of the altered genetic material is necessary and should be compared to that of the non-modified parental organism. Below, we clarify the term "description" and provide you with a more complete list of the required biological information we need in order to process an application.

Also note that under 7 CFR 340.4 (b)(6), and Item 13 (c) of the permit application, you are to describe the molecular biology of the system, including the donor organism. This can be done in the same format as for notifications as shown in the *User's Guide for Notification* at: http://www.aphis.usda.gov/brs/usergd.html.

A description or plasmid map of the base vector should also be included, along with a description of how the vector has been disarmed so as not to cause plant disease, as applicable. Be sure to identify donor organisms for all genetic elements inserted, and indicate if any are listed on the amended list of **biological agents or toxins** in the **Agricultural Bioterrorism Act of 2002** interim final rule (see http://www.aphis.usda.gov/vs/ncie/pdf/btarule.pdf) or on the Center for Disease Control (CDC) list of bioterrorism agents or diseases (see http://www.bt.cdc.gov/agent/agentlist.asp). If so, you are advised to contact the relevant persons indicated for APHIS, PPQ or VS, or the CDC for further information before submitting your application.

2.1. Biological Information for All Permits: Release, movement, or importation

In Item 13(b) of the permit application, provide a one to two page description of the gene product and its current or potential use. If the gene product is for therapeutic use, e.g. an antibody or vaccine, please provide the type of antibody, IgG, IgM, etc., and epitope or antigen, and the disease and target component of the immune system (e.g. CD8 cells). If the gene leads to a product intended for an industrial or other non-food/feed use, indicate whether the gene product itself or a down-stream product produced as a result of the gene is the end-use product, and whether they are new to the plant or are commonly found in plants used for food or feed. You should also include a non-confidential statement in laymen's language about the intended use of the product so that we can submit it with our letter to the State Regulatory Official. For example, "the gene products may be intended to treat a human lung disease or a bacterial disease in young farm animals."

Determine if the gene has any sequence homology to known toxicants, allergens or proteins known or likely to harm non target organisms. Identify both the databases and methods used to perform these analyses.

You do not need to submit cited literature but it should be readily available for prompt submission via fax if requested by APHIS to ensure that we meet our designated turn around time.

2.2. Additional Biological Information Specific to Release Permits

Also in item 13(b) of the permit application, compare properties of engineered proteins/enzymes with that of the native molecule. Please provide information such as: Has the cloning procedure, or have you, intentionally altered the amino acid sequence of the protein; and what effect, if any, does this change have on the biological properties of the protein?

In addition, please quantify the amount of gene product in all plant parts (roots, stem, leaves, pollen, and seeds). If the gene product is an enzyme, provide quantitative enzyme activity in all plant parts. If the product of the enzymatic reaction is important for the intended use, quantify the product as well. Describe whether the data was obtained from growth chamber, greenhouse or field-grown plants. Applicants will be required to provide as a supplemental permit condition for permits issued starting January 2004, the levels of gene products in field-grown plants if not already provided.

Provide data on the thermal stability of the gene products and its sensitivity to gastric digestive conditions of mammals and avian species. As an alternative, provide a scientific rationale, including scientific publication or supporting statements from scientists or veterinarians that data from mammals can be tiered to avian species.

If the gene product has some inherent toxic activity, identify the compound(s) and compare toxic levels produced in the engineered plant with that of the known naturally occurring toxic compounds. Address any differences, especially exposure routes (i.e., ground water contamination, foraging by native or domestic animals, pollen dispersal via wind).

Applicants should evaluate whether the engineering has altered, or would be expected to alter, the levels of any naturally occurring toxicant in the plant, the accumulation or release of toxic compounds recovered during phytoremediation, or has affected any property that could impact confinement measures like seed dormancy, pollen viability, etc.

Applicants must assess if the engineered protein will have direct or indirect damaging or toxic effects on non-target organisms associated with all the plant parts or through other exposure routes, including:

- beneficial organisms (insect pollinators, earthworms, bees, lady beetles, etc.);
- foraging birds, rabbits, deer, rodents or other wildlife;
- humans (potential problems associated with handling plant or seed material;

- potential impact on threatened and endangered species (TES); address county- by-county or as appropriate.

To perform the nontarget organism/TES assessment, prepare and submit a TES worksheet electronically in WORD or WORDPERFECT format. Refer to *Enclosure 2* for a sample TES worksheet. As stated in the enclosure, it would be helpful if you can do the TES assessment for the entire State or for several counties that might have field tests in the future. Lists of TES by state are available at: http://ecos.fws.gov/tess_public/TESSWebpage. When possible, consider the range, habitat, and food of the TES. We strongly encourage the TES worksheet contain no CBI. Please use the headings found in the enclosure.

Provide APHIS with any data that can assist us with the assessment of potential effects on non-target organisms, humans and wildlife, and the environment from exposure as a result of the introduction. Such data may have been submitted to the Food and Drug Administration, the Center for Veterinary Biologics, or other regulatory agency as part of a clinical trial of the product, review of the product for GRAS status or as a food additive, or for industrial use. In addition, state where the product is in the regulatory review process with other regulatory officials.

3. Experimental Design, Confinement, and Supplemental Permit Conditions.

In Items 13(e)–(i) of the permit application form, you must provide a detailed description of the experimental design and confinement measures. Please note that Global Positioning Satellite (GPS) coordinates will be required as a supplemental permit condition for all field test sites to establish the boundaries of the test site containing the regulated articles. Coordinates should be for each corner of the plot, including border rows, if any. APHIS encourages applicants to use a laser range finder to ensure that their isolation distances from sexually compatible plants are accurate. It is prudent to allow extra distance as a safety measure. APHIS inspectors will be using these devices to verify the distances.

Other reproductive confinement measures should be adequately described. For example, describe the frequency of monitoring to ensure pollination bags are intact and/or removal of flowers prior to anthesis, status of flowering period, etc.

For monitoring of crop volunteers, state exactly how many months the field site and fallow zone will be monitored for volunteers, and how the field will be managed to encourage the growth (e.g. through irrigation) and destruction (e.g. through mechanical means or specific herbicide treatment) of volunteers

before flowering. State what actions will be taken if a large number of volunteers are found at the end of the monitoring period.

If you claim these or other confinement measures as confidential, APHIS will write appropriate supplemental permit conditions to ensure that the plants or volunteers will not persist in the environment.

Please be advised that in March 2003, APHIS announced in the Federal Register (see link on page one) revised conditions applicable to permits for these types of introductions regarding the use of dedicated equipment, APHIS-approved SOPs and training programs, record keeping, post-harvest land use restrictions, the size of perimeter fallow zones for all crops, and specific confinement conditions for corn. You should address these in the permit application where applicable. For example, you should state how planting or harvesting equipment will be dedicated to use on the permitted material for the duration of the permit.

APHIS has developed draft criteria for approving the required training programs and SOPs. These are included for your reference as *Enclosures 3* and *4*. You should submit a description of the training program and the SOPs with the permit application, unless APHIS has approved previously-submitted protocols subsequent to the publication of the FR notice. If your training program has already been approved, please submit a list of any new personnel who have or will be trained with regard to the new permit, and the date of actual or proposed training.

In addition to those conditions announced in the FR notice, APHIS supplemental permit conditions also require submission of additional information, most of which has already been included in supplemental permit conditions for field tests of plants expressing pharmaceuticals in the past 3 years. These include:

- a 7 day pre-plant notice and a 21 day pre-harvest notice (however this should also now including notice of intent to move dedicated equipment to other approved sites);
- a 4 week post-planting report that includes: 1) a map of the field site with the GPS coordinates of the plot inclusive of border rows of sexually compatible plants and the location and number of acres of transgenic plants planted for each of the target proteins, 2) the total acreage of the test plot exclusive of border rows, 3) the actual distance from the test plants to the nearest plants of the same crop which will be used for food, feed, or seed production, and 4) the specific confinement option employed if the permit allowed different options;
- a new report submitted after completion of the monitoring period for volunteers of the transgenic crop that includes the dates the field site

and perimeter fallow zone were inspected for volunteers, the number of volunteers observed, and that actions taken to control them.

- the final field data report, as required by APHIS regulations, that must be submitted within 6 months after the termination (harvest or final crop destruct) of the transgenic field trial.

4. Shipments and Container Requirements

If you are intending to transport harvested plant material by truck, car, or rail to a port for export, describe the packaging requirements and name the port. Interstate movements require separate permits.

Our regulations at 7 CFR 340.8 (b) (1 and 2), at: http://www.aphis.usda.gov/brs/7cfr340.html#340.8., describes container requirements for the movement of regulated articles that are plants, plant parts, and seeds. **If you wish to ship in containers that do not meet this regulation, you MUST request a variance from container requirements as described in 7 CFR 340.8 (c) as part of the permit application**. You should describe packaging requirements based on the number or weight of seeds or plant parts, shipping distance and method, construction and capacity of containers, their suitability for the type of plant part being shipped, how they are sealed, a minimum of two levels of containment where each level can independently contain the regulated article in the event of a breach, and how shipments are tracked to ensure arrival at the intended destination.

5. **Formatting the Permit and Confidential Business Information**.

All pages in the permit submission must be numbered. Do not use colored ink in printing because it will not photocopy well. Since most submissions contain CBI, applicants should always follow the guidance for submitting this information, which can be found at: http://www.aphis.usda.gov/brs/pdf/instruction.pdf.

If you have any questions about any points raised in this letter or need assistance in completing your TES worksheet please contact:

Jim White, Branch Chief of Plant Risk Assessment, BRS at (301) 734-5940, or via E-mail at: James.L.White@aphis.usda.gov,

or

Susan Koehler, Branch Chief of Environmental and Ecological Analysis, BRS at Area Code (301) 734-4886, or via E-mail at: Susan.M.Koehler@aphis.usda.gov.

Sincerely,

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services

Enclosures

This application is authorized by the Federal Plant Pest Act (7 U.S.C. 150aa et seq. and the Plant Quarantine Act (7 U.S.C. 151 et seq.)). The information will be used to determine eligibility to receive all types of permits. No permit shall be issued until this application has been approved.

See reverse side for additional information

FORM APPROVED
OMB NO. -579-0085

U.S. DEPARTMENT OF AGRICULTURE
BIOTECHNOLOGY, BIOLOGICS, AND ENVIRONMENTAL PROTECTION

APPLICATION FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

INSTRUCTIONS: Complete this form and enclose the supporting materials listed on the reverse side. See page 3 for detailed instructions.

1. NAME AND ADDRESS OF APPLICANT

2. PERMIIT REQUESTED ("X" one)

- ☐ Limited - Interstate Movement
- ☐ Limited - Importation
- ☐ Release into the Environment
- ☐ Courtesy Permit

3. THIS REQUEST IS ("X" one)

- ☐ New
- ☐ Renewal
- ☐ Supplemental

4. TELEPHONE NUMBER

Area Code ( )

5. MEANS OF MOVEMENT

- ☐ Mail
- ☐ Common Carrier
- ☐ Baggage or Handcarried

By whom _____

6. GIVE THE FOLLOWING (IF APPLICABLE) (IF MORE SPACE IS NEEDED, ATTACH ADDITIONAL SHEET)

	Scientific Name	Common Name	Trade Name	Other Designation
a. Donor Organism:				
b. Recipient Organism:				
c. Vector or Vector Agent:				
d. Regulated Organism or Product:				
e. If product, list names of constituents:				

7. QUANTITY OF REGULATED ARTICLE TO BE INTRODUCED AND PROPOSED SCHECULE AND NUMBER OF INTRODUCTIONS

8. DATE (or inclusive dates of period) OF IMPORTATION, INTERSTATE MOVEMENT, OR RELEASE

9. COUNTRY OR POINT OF ORIGIN OF THE REGULATED ARTICLE

10. PORT OF ARRIVAL, DESTINATION OF MOVEMENT, OR SPECIFIC LOCATION OF RELEASE

11. ANY BIOLOGICAL MATERIAL *(e.g., culture medium, or host material)* **ACCOMPANYING THE REGULATED ARTICLE DURING MOVEMENT**

12. APPLICANTS FOR A COURTESY PERMIT - STATE WHY YOU BELIEVE THE ORGANISM OR PRODUCT DOES NOT COME WITHIN THE DEFINITION OF A REGULATED ARTICLE

13. SEE REVERSE SIDE

I hereby certify that the information in the application and all attachments is complete and accurate to the best of my knowledge and belief.

False Statement: Falsification of any item on this application may result in a fine of not more than $10,000 or imprisonment for not more than 5 years or both. (18 U.S.C. 1001)

14. SIGNATURE OF RESPONSIBLE PERSON	15. PRINTED NAME AND TITLE	16. DATE

FOR APHIS USE ONLY

State Nofitication Letter Sent		State Review Received	Permit Issued ☐ Yes ☐ No
Date of Determination	Permit No.	No. of Permit Labels Issued	Supplemental Conditions Enclosed ☐ Yes ☐ No
Signature of BBEP Official		Date	Expiration Date

APHIS FORM 2000 (JUL 89) *Replaces PPQ Form 1001 which may be used.*

	ENCLOSURES	ENCLOSED ("X")	IF PREVIOUSLY SUBMITTED, LIST DATE & PERMIT NO.
a.	Names, addresses, and telephone numbers of the persons who developed and/or supplied the regulated article.		
b.	A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the nonmodified parental organism (e.g., morphological or structural characteristics, physiological activities and processes, number of copies of inserted genetic material and the physical state of this material inside the recipient organism (integrated or extrachromosomal), products and secretions, growth characteristics).		
c.	A detailed description of the molecular biology of the system (e.g., donor-recipient-vector) which is or will be used to produce the regulated article.		
d.	Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed and produced.		
e.	A detailed description of the purpose of the introduction of the regulated article including a detailed description of the proposed experimental and/or production design.		
f.	A detailed description of the processes, procedures, and safeguards which have been used or will be used in the country of origin and in the United States toprevent contamination, release, and dissemination in the production of the: donor organism; recipient organism; vector or vector agent; constituent of each reglated article which is a product; and regulated article.		
g.	A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location).		
h.	A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations.		
i.	A detailed description of the proposed method of final disposition of the regulated article.		

INSTRUCTIONS

This application form may be used to apply for a limited permit for interstate movement or importation; a permit for release into the environment; or a courtesy permit.

Two copies of this application must be submitted to the Animal and Plant Health Inspection Service, Biotechnology Regulatory Services, Unit 147, Attention: Permits Branch, 4700 River Road, Riverdale, Maryland 20737.

Each copy of the application must be signed by the "responsible person". The responsible person is the person who has control and will maintain control over the introduction of the regulated article and assure that all conditions contained in the permit and regulations in 7 CFR part 340 are complied with. A responsible person must be a resident of the United States or designate an agent who is a resident of the United States.

Confidential Business Information

If there are portions of the application deemed to contain trade secret or confidential business information (CBI), each page of the application containing such information should be marked "CBI Copy". In addition, those portions of the application which are deemed "CBI shall be so designated. The second copy shall have all such CBI deleted and shall be marked on each page of the application where CBI was deleted, "CBI Deleted". If an application does not contain CBI, the first page of both copies shall be marked "No CBI".

Limited Permit for Interstate Movement or Importation

The responsible person seeking a limited permit for interstate movement or importation must complete all items on the application EXCEPT 12, 13(a), (b), (e), and (f).

a. **Interstates Movement** – The responsible person may apply for a single limited permit for the interstate movement of multiple regulated articles in lieu of submitting an application for each individual interstate movement. Each limited permit issued shall be valid for 1 year from the date of issuance. If a permit is sought for multiple interstate movements between contained facilities, the responsible person shall specify in the permit application all the regulated articles to be moved interstate; the origins and destinations of all proposed shipments; a detailed description of all the destinations (contained facilities) where regulated articles will be utilized; and a description of the containers that will be used to transport the regulated articles. A limited permit for interstate movement of a regulated article shall only be valid for the movement of those regulated articles moving between those locations specified in the application. If a person seeks to move regulated articles other than those specified in the application, or to locations other than those listed in the application, a supplemental application shall be submitted to Biotechnology Regulatory Services, Permits Branch. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container. The responsible person who ships a regulated article interstate shall keep records for 1 year to demonstrate that the regulated article arrived at its intended destination.

b. **Importation** – The responsible person seeking a limited permit for the importation of a regulated article shall submit an application for a permit prior to the importation of EACH shipment of regulated articles. The responsible person importing a regulated article shall keep records for 1 year demonstrating that the regulated article arrived at its intended destination.

Permit for Release into the Environment

The responsible person seeking a permit for release into the environment of a regulated article should complete this form in its entirety by submitting data called for by items (1) – (16).

Courtesy Permit

The responsible person seeking a courtesy permit for the introduction (importation, interstate movement, or release into the environment) of genetically engineered organisms not subject to regulation under Part 340 must complete items (1) through (4), (6), (12), (13 (b)), and (15) through (16).

ENCLOSURE 2
SAMPLE TES WORKSHEET

RECIPIENT ORGANISM: Tobacco mosaic virus in tobacco

PRODUCT:
Alpha galactosidase produced in the intracellular spaces of the plant or be wound induced. Alpha galactosidase is an enzyme used for the treatment of Fabry's disease. This serious heritable disease arises from a genetic deficiency in the gene for alpha galactosidase.

LOCATION OF FIELD TEST:
Land used for agriculture for more than two decades. Although plants will be topped (flowers removed), an occasional flower may be produced between required inspections of the field by company employees. Leaves will be harvested six to eight weeks after infection and plants will be allowed to regenerate and leaves harvested six to eight weeks later. The nearest nonagricultural water is more than one mile from the field site. This excludes farm "ponds".

Routine agricultural practices will be performed.

Nearest tobacco field is ½ mile from the field site.

LEVELS PRODUCED AND TISSUE;
One mg of enzyme per gram fresh weight of leaves (greenhouse grown plants). Levels detected in field are lower since plants are more stressed. Less than 0.01mg of enzyme per gram fresh weight is detected in roots, pollen, and stems.

ASSESSMENT
Based on literature review and discussion with tobacco scientists, we can identify no organisms, except plant pests and possibly skunks, that consume tobacco tissues. Earthworms are negatively impacted by nicotine production in the soil.

Even though tobacco is mainly insect pollinated, a significant impact on pollinator species is not expected for the following reasons: they are likely to be killed by pesticide applications and the protein has no toxic activity.

Based on the literature the enzyme is not known to be toxic. It is produced in the roots and leaves, and the levels of product produced is known.

Tobacco is not sexually compatible with any TES plant.

Any unexpected effects from a field test would be minimal by virtue of being confined to the area within the field site (about five acres per plot - six sites per year).

CONCLUSION:
Since there is no identifiable direct effect of this field test on any wild plant or animal species, there is no adverse effect to any threatened or endangered species.

KENTUCKY TES FROM
http://ecos.fws.gov/servlet/TESSWebpageUsaLists?state=KY accessed March 10, 2003

Animals—38

Status	
E	Bat, gray (Myotis grisescens)
E	Bat, Indiana (Myotis sodalis)
E	Bat, Virginia big-eared (Corynorhinus (=Plecotus) townsendii virginianus)
E	Bean, Cumberland (pearlymussel) Entire Range; except where listed as Experimental Populations (Villosa trabalis)
XN	Bean, Cumberland (pearlymussel) AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (Villosa trabalis)
E	Blossom, tubercled (pearlymussel) Entire Range; except where listed as Experimental Populations (Epioblasma torulosa torulosa)
XN	Blossom, tubercled (pearlymussel) AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (Epioblasma torulosa torulosa)
E	Catspaw (=purple cat's paw pearlymussel) Entire Range; except where listed as Experimental Populations (Epioblasma obliquata obliquata)
XN	Catspaw (=purple cat's paw pearlymussel) AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (Epioblasma obliquata obliquata)
E	Clubshell Entire Range; except where listed as Experimental Populations (Pleurobema clava)
E	Combshell, Cumberlandian Entire Range; except where listed as Experimental Populations (Epioblasma brevidens)
XN	Combshell, Cumberlandian AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (Epioblasma brevidens)
T	Dace, blackside (Phoxinus cumberlandensis)
E	Darter, duskytail Entire (Etheostoma percnurum)
E	Darter, relict (Etheostoma chienense)
T	Eagle, bald (lower 48 States) (Haliaeetus leucocephalus)
E	Elktoe, Cumberland (Alasmidonta atropurpurea)
E	Fanshell (Cyprogenia stegaria)
E	Mapleleaf, winged (mussel) Entire; except where listed as experimental populations (Quadrula fragosa)
E	Mucket, pink (pearlymussel) (Lampsilis abrupta)

E	Mussel, oyster Entire Range; except where listed as Experimental Populations (Epioblasma capsaeformis)
XN	Mussel, oyster AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (Epioblasma capsaeformis)
E	Pearlymussel, cracking Entire Range; except where listed as Experimental Populations (Hemistena lata)
E	Pearlymussel, dromedary Entire Range; except where listed as Experimental Populations (Dromus dromas)
E	Pearlymussel, littlewing (Pegias fabula)
E	Pigtoe, rough (Pleurobema plenum)
E	Pimpleback, orangefoot (pearlymussel) (Plethobasus cooperianus)
T	Plover, piping (except Great Lakes watershed) (Charadrius melodus)
E	Pocketbook, fat (Potamilus capax)
E	Puma (=cougar), eastern (Puma (=Felis) concolor couguar)
E	Riffleshell, northern (Epioblasma torulosa rangiana)
E	Riffleshell, tan (Epioblasma florentina walkeri (=E. walkeri))
E	Ring pink (mussel) (Obovaria retusa)
E	Shiner, palezone (Notropis albizonatus)
E	Shrimp, Kentucky cave (Palaemonias ganteri)
E	Sturgeon, pallid (Scaphirhynchus albus)
E	Tern, least (interior pop.) (Sterna antillarum)
E	Wartyback, white (pearlymussel) (Plethobasus cicatricosus)

Plants— 9

Status	Listing
T	Potato-bean, Price's (Apios priceana)
E	Rock-cress, Braun's (Arabis perstellata)
E	Sandwort, Cumberland (Arenaria cumberlandensis)
T	Rosemary, Cumberland (Conradina verticillata)
T	Sunflower, Eggert's (Helianthus eggertii)
T	Goldenrod, white-haired (Solidago albopilosa)
E	Goldenrod, Short's (Solidago shortii)
T	Spiraea, Virginia (Spiraea virginiana)
E	Clover, running buffalo (Trifolium stoloniferum)

ENCLOSURE 3
CRITERIA FOR APHIS APPROVED TRAINING PROGRAMS

The *Federal Register* Notice (68 FR 11337-11340) on *Field Testing of Plants Engineered to Produce Pharmaceutical and Industrial Compounds* states in Section II.1.G that "APHIS will require the permittee to implement an approved training program to ensure that personnel are prepared to successfully implement and comply with permit conditions." The criteria and process that APHIS proposes to use to approve these training programs are described below.

Criteria that APHIS proposes to use to approve such training programs

1) The training program should be applicable to all personnel who handle, store, perform field or laboratory activities with regulated articles or transport regulated articles, or who are responsible for regulatory affairs.
2) Personnel receive instruction and understand APHIS laws, regulations and policies applicable to the introduction of regulated articles pertaining to the duties for which they are responsible. This should include:
   a) The regulations at 7 CFR 340 encompassing the requirements dealing with permits for release, interstate movement, and importation as applicable to the permittee's activities (in particular, including sections 7 CFR 340.4 (a)-(f), 7 CFR 340.7 and 7 CFR 340.8).
   b) The *FR* Notice (68 FR 11337-11340) on *Field Testing of Plants Engineered to Produce Pharmaceutical and Industrial Compounds* and any guidance on its implementation posted on the APHIS website.
   c) Sections of the Plant Protection Act [7 USC 7734 (Sec. 424)] and APHIS regulations [7 CFR 340.0 (b), 340.4 (f) (7) and 340.4 (f) (8)] that deal with consequences of noncompliance.
3) Personnel should have defined roles and responsibilities. They should be specifically trained and evaluated in those procedures that they are expected to perform before they perform them, or be directly supervised by someone who has received such training while carrying out those responsibilities.
4) All personnel should be made aware of the requirement and procedures for reporting accidental or unauthorized releases of regulated articles and any procedures for remedial action to be taken in event of accidental spills or loss of containment of regulated articles.
5) Personnel are required to read and have access to the permit, the standard and supplemental permit conditions and related SOPs that are necessary for understanding and performing the duties for which they are responsible. In particular, the SOPs should include, but not are not limited to, those for:
   a) Cleaning equipment used for planting or harvesting, tractors and tillage attachments such as disks, plows, harrows and subsoilers, before they are moved off of the field test site and/or returned to general use.
   b) Cleaning equipment or vehicles used to off-load or transport harvested material before they are used and after transport of regulated articles to authorized sites.

c) Cleaning dedicated facilities before they are returned to general use.
d) Cleaning and drying seed (if applicable).

6) Personnel should know who has authorized access to the field test site and other restricted areas. Persons having access should receive training regarding site security and how to prevent unauthorized access to regulated articles.
7) There should be mechanisms for inspecting, monitoring and recording the efforts undertaken to meet the conditions of the permit. For example, this could include monitoring or reporting forms, seed inventory and transport forms, third-party auditing or witness verification that critical procedures were performed according to SOPs and/or permit conditions.
8) The training should cover all aspects related to the permitted activities and conditions at all of the intermediate and final destinations listed in the permit application. For example, these may include, but not be limited to the following:
   a) Receipt and storage of seed for planting, the system for marking and maintaining the identity of seeds and plant lines, and procedures for handling packaging material.
   b) Preplanting
      i) Notification 1 week in advance of the anticipated planting date and site-specific contact information and whether planting equipment will be moved to other authorized sites.
      ii) Verifying that the lines to be planted, the plot layout, location, isolation distance and fallow zones are consistent with the approved permit and conditions.
      iii) Ensuring that the planter is cleaned of any plant material not authorized to be planted at the site.
   c) Planting
      i) Documenting planting date and personnel involved.
      ii) Collecting, recording and submitting information needed for the 28-day post-planting report: GPS coordinates; acreage of test plot; location, number and/or acreage of lines planted for each target protein; confinement method used at each site; and surveying for nearest distance to commercial crop of the same species.
      iii) Performing and verifying planter cleanout according to SOP before moving it off of the field test site and returning it to dedicated storage.
      iv) Destruction, storage or return of unused seed, plantlets or propagules (including container requirements for movement); maintaining chain of custody.
   d) Mid-season
      i) Recording field activities, personnel who performed them, and cleaning and storage of farm implements according to SOPs.
      ii) Keeping fallow area free of volunteers or sexually compatible species.
      iii) Monitoring for and reporting of nontarget effects or other unusual occurrences; procedures for preventing nontarget effects (e.g. rodent, bird or other wildlife management).

iv) Monitoring flowering dates and implementing confinement protocols regarding flowering (such as bagging, flower removal, etc.) and recording how often, when and who performed them.
v) Notification 21 days in advance of the anticipated harvest date, anticipated movement of harvest equipment to other authorized sites, or anticipated cleaning for return of harvest or planting equipment or dedicated facilities to general use.

e) Harvest (or Crop Destruct)
i) Documenting or recording harvest date, personnel involved, harvest inventory, dedicated storage location or transport of harvested material, and chain of custody.
ii) Performing and verifying harvesting equipment cleanout according to SOPs before moving them off-site or returning them to general use.
iii) Dedicated storage of harvesting equipment.
iv) Cleaning and drying of harvested seed and dedicated facilities according to SOPs.

f) Post-Harvest/Post-Season
i) Procedures for destruction, burial or removal of regulated plant material on the field site and fallow zone immediately after harvest, or as soon as possible the following growing season, and record keeping.
ii) Submitting 6-month field data report.
iii) A) Procedures for monitoring and destruction, burial or removal of volunteers on the field site and fallow zone during the designated monitoring period.
B) Recording information associated with this activity for the 3-month post-season monitoring report and submitting the report.
iv) Procedures for planting and harvesting a crop on the field site or fallow zone, if such authorization has been granted.
v) Cleaning and storage of farm implements used on the former field test site and fallow zone according to SOPs.

ENCLOSURE 4

Criteria for APHIS-Approved SOPs Required for Field Tests of Plants Genetically Engineered to Produce Pharmaceuticals and Industrials

REQUIRED SOPS AND RISK CATEGORIES

Supplemental permit conditions and the FR Notice require SOPs for cleaning certain equipment or facilities and for certain operations that handle or come into contact with regulated plant material. These are listed below. Risk categories have been assigned to these pathways based on the potential, volume and frequency with which regulated seed or other plant material can become mixed with non-regulated plant material or for viable material to enter a propagative environment in the absence of appropriate SOPs for cleaning or handling. It also considers the availability of adequate SOPs. The risk categories are defined as:

Low risk*

Medium risk**

High risk***

Very high risk****

****Harvester and ***Planter** : Cleaning before they are moved off the field test site or moved between authorized field test sites on public roads. This equipment must be dedicated to use on authorized sites from the time of planting to the time of harvesting. Before they are returned to general use after the harvest is complete, the permittee must notify APHIS, BRS and the PPQ Regional Biotechnologist and State Regulatory Official at least 21 days in advance of cleaning for this purpose so that APHIS or an APHIS-certified inspector may schedule an inspection to witness and verify that the cleaning meets the standard that all regulated material is removed. SOPs must be submitted and approved for this purpose prior to notice being given to APHIS requesting an inspection.

****Storage facilities for seed and equipment used to handle regulated articles-** Cleaning before they are returned to general use. The permittee must notify APHIS, BRS and the PPQ Regional Biotechnologist and State Regulatory Official at least 21 days in advance of cleaning for this purpose so that APHIS or an APHIS-certified inspector may schedule an inspection to witness and verify that the cleaning meets the standard that all regulated material is removed. SOPs must be submitted and approved for this purpose prior to notice being given to APHIS requesting an inspection

*****Seed cleaning, processing, and drying (seed for planting or other purposes)** of regulated articles. [It is generally thought that these will be contained within the storage facility and will be inspected when that facility is returned to general use.]

Equipment to off-load, haul, or move seed or harvested material, e.g. ***elevators, conveyers, or augers; **trucks and wagons- Cleaning before use and after off-loading or transport of regulated seed or harvested material at an authorized site or location

***Tractors (including attachments for spraying, mowing and tilling)** used in the field test site or fallow zone during the growing period of the regulated article or during the volunteer monitoring period

SOPs SHOULD INCLUDE INFORMATION ON THE FOLLOWING:

Who performs the operation and their qualifications. The person performing the operation must be trained in the APHIS approved SOPs or be supervised by a person trained in the APHIS approved SOPs.

For cleaning harvestors, planters, augers, elevators, and conveyers, the person performing the operation must be specifically trained in the SOP.

Restricted access or use of the equipment. During the time that equipment is being used for the regulated articles and before it is cleaned to be returned to general use, this equipment must be posted as restricted to authorized personnel only. When not is use, it needs to be locked or secured to prevent use by unauthorized personnel.

Where and when equipment is to be cleaned. Equipment cleaning should be performed on the field test site, fallow zone, or other regulated area designated in the permit or SOPs. This will allows for containment or recovery of seed or other propagules, and monitoring for the volunteers in the event that seed enters a propagative environment. If portable equipment cannot be cleaned immediately after use (within 24 - 48 hrs), it should be enclosed in a restricted area or stored in a facility dedicated to the storage of the regulated article and equipment.

Make, model, type, and serial number of the equipment covered by the SOP. This applies to planting and harvesting equipment, equipment used to off-load, haul, or move seed or harvested material [e.g., elevators, augers, trucks, wagons], and seed drying, processing, or cleaning equipment. If no serial number is available, a permanent tag that provides for unique identification should be attached to the equipment. SOPs should be applicable to the make and model of the equipment.

Reference or source of the SOP. Manufacturers instruction manual for cleaning or operation procedures, or other references from agricultural extension or consulting services on cleaning protocols for identity preservation or seed purity.

Step-by-step methods and materials for cleaning.

Harvesters: All areas where seed or other plant material may dislodge from the harvester during transport from the field test site should be cleaned prior to return to the dedicated storage facility storage, or transport to another authorized field test site.

In the latter case APHIS must be notified. Transport in an enclosed truck or covered trailer will most likely be required unless the harvester is thoroughly cleaned per an SOP applicable to the make and model of the equipment [e.g. as specified by the manufacturer]. For return to "general use", the harvester cleaning must be witnessed, inspected, and approved by APHIS or an APHIS-certified inspector.

Planters: Seed should be removed from hoppers and all areas used to deliver seed (e.g. hoses, tubes, gears, planting shoes) and areas in which seed could reside. If the equipment is being moved to another authorized site for use with the same or similar constructs, planter boxes, bins, etc. should be removed or emptied, and the planter cleaned sufficiently to prevent material from falling during transport, or it should be transported in an enclosed truck or trailer. For return to general use, the planter cleaning must be witnessed, inspected and approved by APHIS or an APHIS-certified inspector. SOP must be applicable to the make and model of the equipment.

Buildings used to store dedicated equipment or regulated articles: Methods such as brushing, wet or dry vacuuming, high pressure water or steam cleaning, should be sufficient to remove seed or other plant material and crop residue from equipment, surfaces, floors, storage areas, etc. For return to general use, the building cleaning must be inspected and approved by APHIS or an APHIS-certified inspector.

Seed (or plant material) storage, processing, and drying equipment: Methods should prevent spillage and commingling of plant material with unregulated plant material. Thorough cleaning, (such as brushing, vacuuming of all areas, followed by high pressure hot water, steam cleaning, or compressed air) is required so that NO regulated article or residues of regulated articles remain.

Trucks or wagons used to haul seed or harvested material should be covered or enclosed during transport, and should be swept, brushed, or sprayed out as necessary to remove all regulated plant material from the interior and exterior (including the wheels) after unloading at an authorized facility.

Tractors and other field equipment: Methods should be sufficient to remove seed and other plant material from wheels and attachments before moving off of the test site or authorized areas. This might include brushing, hosing, or high pressure spraying. If spraying equipment is used during the period of viable pollen production by the regulated plants, procedures (e.g. high pressure hot water or steam) need to be taken to assure any pollen adhering to the spraying equipment is removed or devitalized before the equipment is used in a sexually compatible species off the field test site.

Effectiveness of the cleaning method. Data must be presented for cleaning planters and harvesters, and for other equipment such as grain elevators, augers, conveyers, seed cleaners, seed dryers, and seed processors before they are returned to general use unless it can be demonstrated through inspection that the cleaning meets the standards and tolerances required by APHIS. This may require that the equipment can be completely disassembled and observed throughout.

Final disposition of material recovered during cleaning operations. Viable plant material should be destroyed, stored in or returned to facilities, field test sites or other locations authorized in the permit. Non-viable material may be disposed of through appropriate waste disposal methods, depending on the constructs and where the foreign protein is expressed. **No recovered plant material may be directed toward food or feed use.**